EXPRESS MAIL MAILING LABEL

No. EL302645877US

COPY

PATENT
Atty. Docket No. CWP-012FW
(1451/2)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S): DAVID E. CHARLTON and NEAL W. MILLER

SERIAL NUMBER: 07/995,331 GROUP NUMBER: 1817

FILING DATE: Dec. 23, 1992 EXAMINER: Spiegel, C. A.

TITLE: TEST DEVICE AND METHOD FOR COLORED PARTICLE IMMUNOASSAY

CERTIFICATE OF HAND DELIVERY

I hereby certify that this correspondence is being deposited with the receptionist of Art Unit 1817 and Examiner C. Spiegel At the US Patent and Trademark Office, Washington D.C., 20231 on:

Date: , 1997

(Print Name)

The Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

DECLARATION OF DAVID E. CHARLTON, NEAL W. MILLER AND MARGARET MAZZEO UNDER 37 C.F.R. 1.131

We, David E. Charlton, Neal W. Miller and Margaret Mazzeo, hereby declare as follows:

1. Prior to March 27, 1987, we had actually reduced to practice, in the United States, a method for detecting, by visual observation of color development, the presence of a ligand in a liquid sample, i.e., the presence of human chorionic gonadotropin (hCG) in urine, which satisfies all of the limitations of the claimed subject matter in the above-captioned patent application as currently amended. As evidence of our reduction to practice, we attach as "Exhibit A" a true copy (except all dates have been redacted) of a page from a notebook of Margaret Mazzeo (née Verbanic), a scientist employed by the assignee of the instant application, Carter-Wallace, Inc.,

and who performed experiments under the direction of named coinventor Dr. Charlton. These experiments described the use of a device for detecting the ligand hCG according to the presently claimed invention. The original notebook page bears a date earlier than March 27, 1987, and refers to experiments completed as of an earlier date. (The date on the notebook page has been deleted on the copy submitted herewith.)

2. Exhibit A is a date-redacted copy of a notebook page entitled "New Chromatostrip Device-Tested Spiked Problem Urine" and documents a "preliminary evaluation" in which an experimental device was used. The device comprises a housing made of a pair of substantially rigid, water-impervious, rectangular planar plastic strips sandwiching an adsorbent test strip ("Eaton Dickman 111" sorbent material) laminated together by bonding tape. The housing has two windows (viewing holes passing through the top surface permitting visual observation of portions of the test strip), and an inlet (bottom part of the housing where the strip is exposed between the top and bottom housing surfaces). The sorbent strip material defines a flow path extending from the inlet to a test site comprising immobilized antibody specific for hCG (20 µl of a solution of polyclonal antibody coupled to adhered latex particles) and to a control site (adhered latex particles coated with non-specific polyclonal antisera). As Exhibit A indicates, five urine samples were prepared, 250 µl each spiked with 0, 25, 50, and 150 mIU, and 1 IU of hCG. Each urine sample was added to an amount (assay strength $OD_{525} = 0.375$) of lyophilized gold sol-hCG specific antibody ($b105_{15nm}$). Each reconstituted conjugate sample mixture was applied to the inlet of one experimental chromatostrip device as described above and allowed to flow along the flow path by sorption. Exhibit A illustrates the five experimental devices used in this experiment and shows the experimental results. A color is observed at each test site (top window) for samples containing the antigen and it develops darker as the concentration of hCG in the urine samples increases. A pale color is observed at the control site (bottom window) that remains constant in intensity and is independent of the concentration of hCG.

3. Exhibit A records an experiment which constituted a reduction to practice of the invention as currently claimed. The device operated to detect the ligand hCG in a liquid sample in accordance with the claimed method of the invention. To reiterate, the liquid sample suspected

(here known) to contain a ligand (urine with added 0 to 1 IU of hCG) was applied to the inlet of the experimental chromatostrip device described in paragraph 2 above. The test site has immobilized thereon a first binding protein (polyclonal antibody bound to latex particles) having a binding site specific for a first epitope of the ligand (hCG). The conjugate is a colored particle (gold sol of 15 nm diameter) coupled to a binding protein (antibody b105) having a binding site specific for a second epitope on the ligand (hCG). The liquid sample was transported by sorption in the sorbent material along the flow path in admixture with the conjugate. A visually observable color at the test site was produced by aggregation of the colored particulate material. A positive result was observed when the color intensity (darkening) at the test site was greater than at the control site. A negative result was observed when the intensity of the color observed at the test site was less than or equal to the intensity at the control site. Therefore, the presence of the ligand in the sample was detected by visual observation of color development at the test site, as shown in Exhibit A.

4. All statements made herein of our own knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: March 10, 1997 David E. Charlton

David E. Charlton, coinventor

Date: _____

_____

Neal W. Miller, coinventor

Date: _____

_____

Margaret Mazzeo, corroborating scientist

NB- No: 1608-049 PAGE

PURPOSE New Chromatostrip Device ~ Tested spiked problem urine

Materials: 6105 15mm OD525 750 ~ assay strength 375

fresh problem urine (MV)

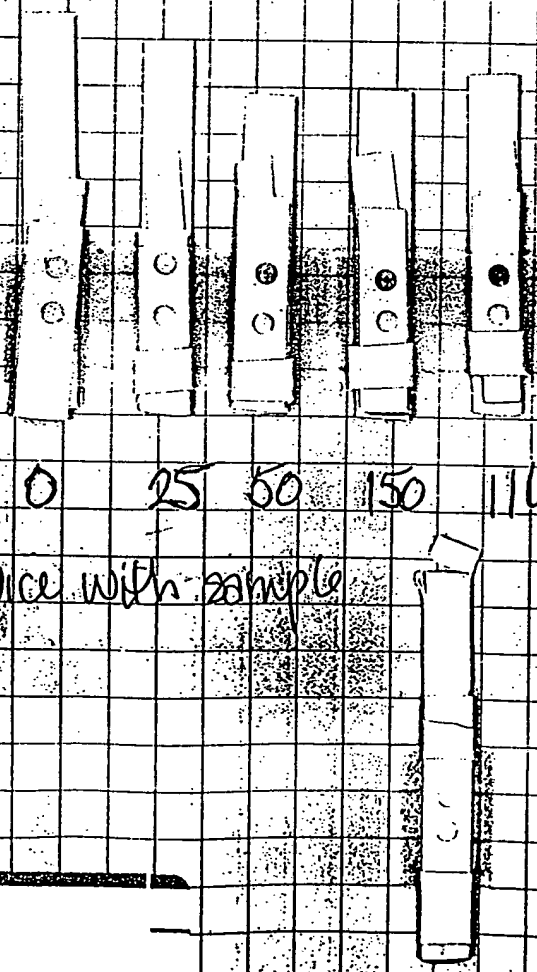
hCG (Sigma lot #)

25 50 150 mIU 1 IU

polyclonal latex CA

20ul spotted on Eaton Dikeman 111

Procedure: reconstituted 5ml lyophilized conjugate with 250ul urine of blank or spiked w/ hCG



0 25 50 150 1IU

Clinicals ~ Device with sample

EXHIBIT A

SIGNED M. Verbanic DATE

WITNESSED BY DATE

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Date: _____

_____
David E. Charlton, coinventor

Date: MARCH 10, 1997

_____
~~Neal~~ W. Miller, coinventor
NEIL

Date: _____

_____
Margaret Mazzeo, corroborating scientist

DATE | EXPERIMENT NB- | BOOK NO. | PAGE

PURPOSE

New Chromatostrip Device ~ Tested spiked problem urine

Materials: b105 15nm OD525 760 ~ assay strength .375

fresh problem urine (MM)

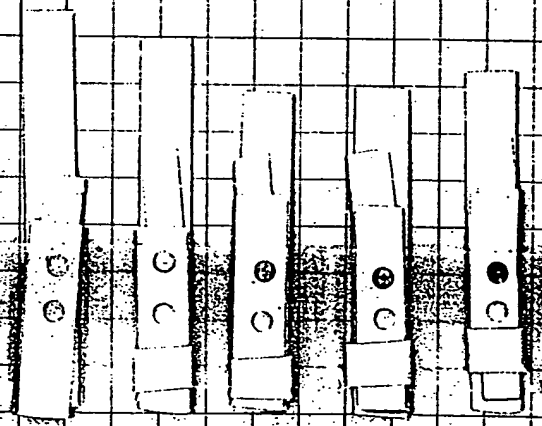
hCG (Sigma lot #)

25 50 150 mIU 1 IU

polyclonal latex CA

@ 20ul spotted on Eaton Dikeman 111

Procedure: reconstituted dry lyophilized conjugate with 250ul urine or blank or spiked w/ hCG



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Clinicals ~ Device with sample

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Date: _____ _____
David E. Charlton, coinventor

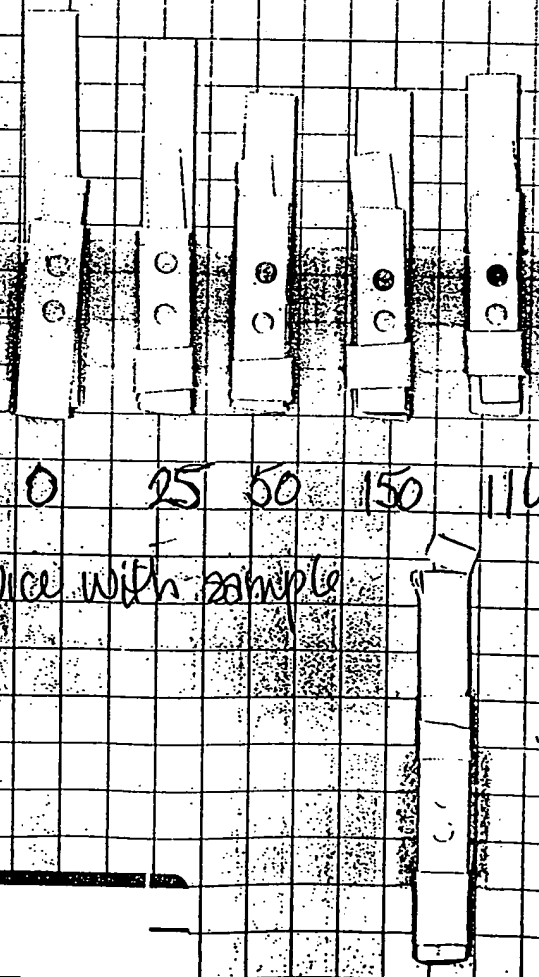
Date: _____ _____
Neal W. Miller, coinventor

Date: March 5, 1997 Margaret Mazzeo
Margaret Mazzeo, corroborating scientist

PURPOSE New Chromatomp Device ~ Tested spiked problem urine

Materials: blOS 15nm OD525 750 ~ assay strength .375
fresh problem urine (MV)
hCG (Sigma lot #)
25 50 150 mIU 1 IU
polyclonal latex CA
20ul spotted on Eaton Dikeman 111

Procedure: reconstituted std lyophilized conjugate with 250ul urine or blank or spiked w/ hCG



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Clinicals ~ Device with sample

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